MYCOTAXON

Volume 110, pp. 325–330 *October–December 2009*

First record of *Mattirolomyces terfezioides* from the Iberian Peninsula: its southern- and westernmost locality

Gábor M. Kovács[1*], María P. Martín[2] & Francisco D. Calonge[2]

**gmkovacs@caesar.elte.hu*
[1]Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University
Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary

[2]Departamento de Micología, Real Jardín Botánico, CSIC
Plaza de Murillo 2, 28014 Madrid, Spain

The paper honors the 150th anniversary of the birth of László Hollós (1859-1940).

Abstract — During revision of *Terfezia* specimens in the Mycological Collection of the Herbarium of the Real Jardín Botánico of Madrid (MA-Fungi), morphological and molecular analyses revealed a specimen collected in Madrid as the truffle *Mattirolomyces terfezioides*. This paper presents the first record of the species from the Iberian Peninsula, the western- and southernmost known locality for *M. terfezioides* in Europe. General characteristics of known collection sites are also discussed.

Key words — *Pezizaceae*, *Hydnobolites*, biogeography, host plant, ITS

Introduction

The truffle *Mattirolomyces terfezioides* (Mattir) E. Fisch. 1938 was described as *Choiromyces terfezioides* by Mattirolo based on a northern Italian collection from Piemonte (Mattirolo 1887). Fischer (1938) determined that it represented a distinct genus, which he named *Mattirolomyces* for "the Mattirolo fungus." Trappe (1971) placed *Mattirolomyces* into the desert-truffle genus *Terfezia* but changed its rank to a subgenus. Subsequent molecular phylogenetic analyses unambiguously show that *Mattirolomyces* merits generic status (Percudani et al. 1999, Díez et al. 2002, Læssøe & Hansen 2007) within the *Pezizaceae*, lineage A (Læssøe & Hansen 2007, Trappe et al. 2009). Originally the genus was monotypic and represented only by *M. terfezioides*. Healy (2003) later described a new species, *M. tiffanyae* Healy, but according to molecular phylogenetic analyses it does not belong to *Mattirolomyces* (Hansen, Healy & Kovács, unpublished data). However, a more recent study on Australian and

Kalahari desert truffles has revealed two other *Mattirolomyces* species (Trappe et al. 2009), and one more is being described from North America and Asia (Kovács, Alsheikh & Trappe, unpublished data). These recent findings establish the genus *Mattirolomyces* as more diverse taxonomically and more widely distributed than previously thought.

Mattirolomyces terfezioides was described with a collection from Testona, Moncalieri-Piemonte, Italy as the holotype. The species was subsequently collected in other North Italian localities (Montecchi & Sarasini 2000). The species has been recorded from Provence, 18 km east of Avignon in Southern France (OSC Trappe 4548, Alsheikh 1994) as well as from the northern Balkan Peninsula (Ławrynowicz et al. 1997). The first record of the species from Hungary was in 1915 (Hollós 1933); most Hungarian collections are from mixed black-locust (*Robinia pseudoacacia*) forests on sandy soils (Szemere 1965, Babos 1981, Király et al. 1992, Király & Bratek 1992, Kovács & Bagi 2001, Kovács et al. 2001, 2007).

During comparative studies of *Terfezia* species in the Mycological Collection of the Herbarium of the Real Jardín Botánico, Madrid (MA-Fungi), a possibly mis-identified specimen was found among the *Terfezia* specimens. Our main aim in this paper is to present the morphological features and molecular taxonomic results that reveal it as the first record of *M. terfezioides* from the Iberian Peninsula. General characteristics of the habitats of the species are also discussed.

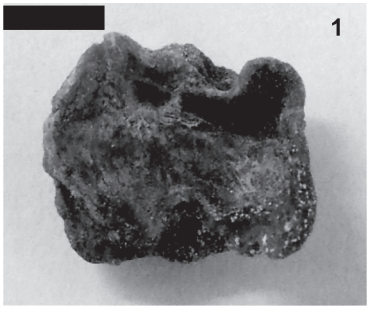
Materials and methods

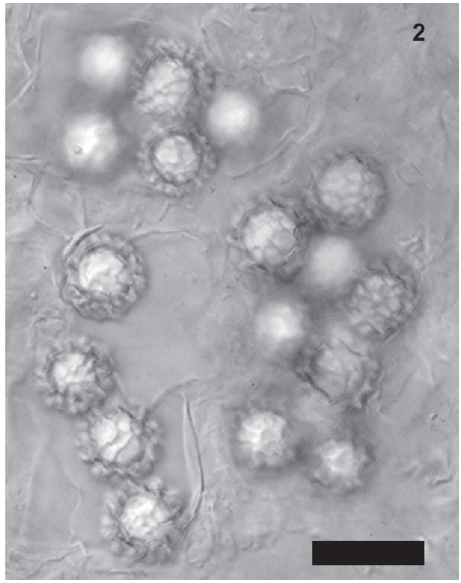
Microscopy

The light microscopic characterization of the dry specimen was carried out by studying hand made sections of the ascoma after rehydration in 5% KOH. Spores and ascus sizes were measured with an ocular-micrometer. Micrographs were obtained using Nomarski (differential interference contrast) optics of a Nikon 80i microscope equipped with digital camera. Ascospores were studied by scanning electron microscopy with a Hitachi S-3000N SEM (Madrid, RJB) after gold coating of the samples.

Molecular identification

A small piece (~ 15 mg) of the dry ascoma was used to extract total genomic DNA with an E.Z.N.A. Fungal DNA Extraction kit (Omega) following the manufacturer's instructions with small modifications. The ITS region of the nrDNA was amplified and sequenced as detailed by Martín & Winka (2000). The electrophoregrams were checked and assembled by use of the Staden Program Package (Staden et al. 2000). BLASTn (Altshul et al. 1990) was used to compare our sequences with nuclear sequences deposited in public databases. Our ITS sequence has been deposited in GenBank (GQ422438).





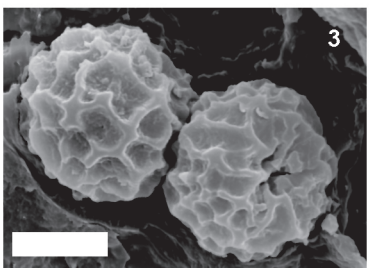


Fig. 1. The slice of the small ascoma of *Mattirolomyces terfezioides* in the herbarium specimen MA-Fungi 8212 collected in Madrid, Spain. Bar = 5 mm. Fig. 2. Light microscopy of ascospores of *M. terfezioides* (MA-Fungi 8212). Bar = 20 µm. Fig. 3. Scanning electron micrograph of ascospores of MA-Fungi 8212. Bar = 10 µm.

Results and discussion

Specimen MA-Fungi 8212 was collected in Madrid city (in "Colonia del Retiro") on 30 August 1984 and deposited in the herbarium as *Terfezia claveryi* Chatin 1892. A penciled note with the specimen states (translated from Spanish): "Obs. it has reticulate spores". In 1990 the specimen was studied again and still marked as *Terfezia claveryi*. During a further study in 2001, the specimen was marked as *Hydnobolites cerebriformis* Tul. & C. Tul. 1843. No further information either about the locality or the environment was recorded with the specimen.

MA-Fungi 8212 contains a relatively thick slice of a small ascoma (Fig 1). The material was in poor condition: one side of the slice was moldy. The organization of the gleba was hardly visible, and no distinct peridium could be detected even in the microscope slides: only crushed, broken cells could be seen. Most asci were broken, but spores were clustered in groups of eight. Spores were relatively small, 15–18 µm broad (ornamentation included) and reticulate (Figs 2 and 3), and several were broken. The anatomical features of the specimen fit the known characteristics of *M. terfezioides* (Szemere 1965,

Alsheikh 1994). Nevertheless, due to the poor development and condition of the specimen and its geographic origin (see below), one could easily think that one of the two truffle species written on the capsule was found.

The ITS sequence of the specimen was identical with *M. terfezioides* ITS sequences deposited in GenBank. The previously sequenced nrDNA ITS region of the *M. terfezioides* collected in Provence, France (GQ231754, Trappe et al. 2009), was also identical with *M. terfezioides* GenBank sequences and that of the Madrid specimen. Almost no variation was found between the nrDNA ITS sequences of *M. terfezioides* from various localities in Hungary and Italy (Kovács et al. 2001). This invariability enabled the design of species-specific PCR primers used to identify the natural hosts of *M. terfezioides* (Kovács et al. 2007).

Although most *M. terfezioides* records are from mixed *Robinia pseudoacacia* forests on sandy soils in the Danube basin, the urban environment of the Madrid collection is not unique for the species. The first documented collection of the fungus in Hungary by László Hollós was in a cemetery of Szekszárd "below an old black-locust tree at a weakly grassed almost bare place" on 27 July 1915 (Hollós 1933). This was the first published record of the fungus since the species was originally described from Piemonte. Babos (1981) reported herbarium specimens collected within Budapest on sandy soils, even in a cemetery, with *Robinia* and other trees, and she also noted a collection from a village garden close to Budapest. One of us (G.M. Kovács) also collected the fungus in a cemetery in the small village Felsőszentiván, close to the Southern border of Hungary in early November 2004. Montecchi & Sarasini (2000) also reported various cultivated environments (e.g., *Ficus* stumps, asparagus market garden) as *M. terfezioides* habitats – similar to Mattirolo (1887) who mentioned that people regularly collected *M. terfezioides* during cultivation.

In light of these data, it is not odd that *M. terfezioides* was collected in Madrid in the "Colonia del Retiro" district. Unfortunately no information was recorded about the soil in which it was found or the plants that surrounded it. In view of the collections listed above and further details surrounding the biotic environments of the known host plants (Kovács et al. 2007), we infer that *M. terfezioides* has no specific host.

Until now the westernmost known locality of *M. terfezioides* has been southern France. Its easternmost known locality is in Hungary. Madrid, at 40°24' N and 3°41' W, represents the most western and southern known locality of the species. Moreover, this is the first record of *M. terfezioides* from the Iberian Peninsula. As the hypogeous fungi of Spain have been widely studied and both the harvesting and the cultivation of truffles and desert truffles have a long tradition (Moreno et al. 1986, 1991; Honrubia et al. 1992; Alvarez et al. 1993; Moreno-Arroyo et al. 1999, 2005; Calonge et al. 1999, 2000; Díez et al.

2002; Morte et al. 2008), we might assume that the species might be rare in the area. Nevertheless, one should bear in mind the unsuspected geographic presence of *M. terfezioides* and its similarities with other hypogeous fungal species, especially when poorly developed or degraded ascomata are found: the species might be overlooked and/or misidentified as happened in the case of MA-Fungi 8212.

Acknowledgments

The valuable presubmission reviews of Prof. James M. Trappe (Oregon State University, Oregon, USA) and Prof. Gabriel Moreno (University of Alcalá de Henares, Spain), the help of Kinga Sata (Budapest, Hungary) in translating Italian texts, and the SEM technical assistance of Miguel Jerez (RJB, Madrid) are greatly appreciated. The research was supported by the SYNTHESYS Project, EU (ES-TAF-3437), the project Flora Mycologica Iberica (CGL2006-12732-CO2-01/BOS) and the Hungarian Research Fund (OTKA K72776).

References

Alsheikh AM. 1994. Taxonomy and mycorrhizal ecology of the desert truffles in the genus *Terfezia*. PhD Diss, Oregon State University, Corvallis.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Alvarez IF, Parlade J, Trappe JM, Castellano MA. 1993. Hypogeous mycorrhizal fungi of Spain. Mycotaxon 47: 201–217.

Babos M. 1981. Distribution of *Choiromyces venosus* and T*erfezia terfezioides* in Hungary. (in Hungarian with English summary) Mikol. Közl. 20(1–2): 47–56.

Calonge FD, Vasco F, Fernández A. 1999 Contribución al conocimiento de los hongos hipogeos de Albacete, España. Bol. Soc. Micol. Madrid 24: 187–191.

Calonge FD, Fernández A, Vasco F. 2000. Contribución al conocimiento de los hongos hipogeos de Albacete (España). II. Registro de 10 especies nuevas para la provincia. Bol. Soc. Micol. Madrid 25: 171–176.

Díez J, Manjón JL, Martin F. 2002. Molecular phylogeny of the mycorrhizal desert truffles (*Terfezia* and *Tirmania*), host specificity and edaphic tolerance. Mycologia 94: 247–259.

Fischer E. 1938. Klasse *Ascomycetes,* Reihe *Euascales.* Unterreihe VIII. *Tuberineae.* In: Engler A, Harms H (eds), Natürlichen Pflanzenfamilien V. Wilhelm Engelmann Verlag, Leipzig, p 39

Healy RA. 2003. *Mattirolomyces tiffanyae*, a new truffle from Iowa, with ultrastructural evidence for its classification in the Pezizaceae. Mycologia 95: 765–772.

Hollós L. 1933. Szekszárd vidékének gombái (Fungi regionis Szekszárdiensis). (in Hungarian) Math. Term. Tud. Közl. 37(2): 1–205.

Honrubia M, Cano A, Molina-Niñirola C. 1992. Hypogeous fungi from southern Spanish semiarid lands. Persoonia 14: 647–653.

Király I, Bratek Z. 1992. *Terfezia terfezioides* a common truffle in Hungary. Micol. Veg. Med. 7: 43–45.

Király I, Bratek Z, Albert L, Lukács Z. 1992. The arenicolous truffle (*Terfezia terfezioides*). (in Hungarian with English summary) Mikol. Közl. 31(1–2): 49–54.

Kovács GM, Bagi I. 2001. Mycorrhizal status of a mixed deciduous forest from the Great Hungarian Plain with special emphasis on the potential mycorrhizal partners of *Terfezia terfezioides* (Matt.) Trappe. Phyton 41: 161–168.

Kovács GM, Rudnóy S, Vágvölgyi C, Lásztity D, Rácz I, Bratek Z. 2001. Intraspecific invariability of the internal transcribed spacer region of rDNA of the truffle *Terfezia terfezioides* in Europe. Folia Microbiol. 46: 423–426.

Kovács GM, Jakucs E, Bagi I 2007. Identification of host plants and description of sclerotia of the truffle *Mattirolomyces terfezioides.* Mycol. Progress 6: 19–26.

Læssøe T, Hansen K. 2007. Truffle trouble: what happened to the *Tuberales?* Mycol. Res. 111: 1075–1099.

Ławrynowicz M, Marković M, Milenković M, Ivančević B. 1997. *Terfezia terfezioides* – a new hypogeous fungus for Balkan Peninsula. Acta Mycol. 32: 233–238.

Mattirolo O. 1887. Illustrazione di tre nuove specie di Tuberacee Italiane. Mem. della Reale Accad. Sci. Torino (Seconda serie) 38: 377–393.

Martín MP, Winka K. 2000. Alternative methods of extracting and amplifying DNA from lichens. Lichenologist 32: 189–196.

Montecchi A, Sarasini M. 2000. Funghi Ipogei d'Europa. Associazione Micologica Bresadola, Fondazione Centro Studi Micologici, Trento.

Moreno G, Galan R, Ortega A. 1986. Hypogeous fungi from continental Spain. 1. Crypt. Mycol. 7: 201–229.

Moreno G, Galan R, Ortega A. 1991. Hypogeous fungi from peninsular Spain. 2. Mycotaxon 42: 201–238.

Moreno-Arroyo B, Calonge FD, Gómez J, Pulido E. 1999. Flora micológica hipogea de Andalucía (España). Bol. Soc. Micol. Madrid 24: 127–178.

Moreno-Arroyo B, Gómez J, Pulido E. 2005. Tesoros de nuestro montes. Trufas de Andalucía. Consejería de Media Ambiente, Junta de Andalucía, Córdoba, Spain.

Morte A, Honrubia M, Gutiérrez A. 2008. Biotechnology and cultivation of desert truffles. In: Varma A (ed.) Mycorrhiza, State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics. Springer-Verlag, Berlin, Heidelberg, pp: 467–483.

Percudani R, Trevisi A, Zambonelli A, Ottonello S. 1999. Molecular phylogeny of truffles (*Pezizales: Terfeziaceae, Tuberaceae*) derived from nuclear rDNA sequence analysis. Mol. Phyl. Evol. 13: 169–180.

Staden R, Beal KF, Bonfield JK. 2000. The Staden package, 1998. Methods Mol. Biol. 132: 115–130.

Szemere L. 1965. Die unterirdischen Pilze des Karpatenbeckens (Fungi hypogaei territorii Carpato-Pannonici). Akadémiai Kiadó, Budapest, Hungary.

Trappe JM. 1971. A synopsis of the *Carbomycetaceae* and *Terfeziaceae* (*Tuberales*). Trans. Brit. Mycol. Soc. 57: 85–92.

Trappe JM, Kovács GM, Claridge AW. (2010, in press). Comparative taxonomy of desert truffles of the Australian Outback and African Kalahari. Mycol. Progress.